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Published:

— with international search report

(88) Date of publication of the international search report:  
13 July 2006

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: MODIFIED POLYNUCLEOTIDES FOR USE IN RNA INTERFERENCE

(57) Abstract: Methods and compositions for performing RNA interference comprising a wide variety of stabilized siRNAs suitable for use in serum-containing media and for in vivo applications, such as therapeutic applications, are provided. These siRNAs permit effective and efficient applications of RNA interference to applications such as diagnostics and therapeutics through the use of one or more modifications including orthoesters, terminal conjugates, modified linkages and 2'modified nucleotides. Uniquely modified siRNAs have been developed that reduces off-target effects incurred in gene-silencing. The modifications include phosphorylation of the first 5' terminal antisense nucleotide; 2' carbon modifications of the first and second or first, second, and third 5' terminal antisense nucleotides; and optionally 2' carbon modifications of the first and second or first, second, and third 5' terminal sense nucleotide. Control and exaequo molecules are also provided. siRNA molecules and related control, trackability and exaequo agents with specific stability modifications were developed.

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/10343

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07H 21/04; A61K 48/00

US CL : 536/24.5; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/24.5; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,506,559 B1 (FIRE et al) 14 January 2003 (14.01.2003), see entire document.	1
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Y		2-5 and 10-13
X	AMARZGUIOUJ et al. Tolerance for mutations and chemical modifications in a siRNA. Nucleic Acids Research. 2003, Vol. 31, No. 2, pages 589-595, see entire document.	1-5, 10
P, X	CHIU et al. siRNA function in RNAi: A chemical modification analysis. RNA. 2003, Vol. 9, No. 9, pages 1034-1048, see entire document.	1-5 and 8-13
P, X	CZAUDERNA et al. Structural variations and stabilising modifications of synthetic siRNAs in mammalian cells. Nucleic Acids Research. 2003, Vol. 31, No. 11, pages 2705-2716, see entire document.	1-5 and 8-10
X	PARRISH et al. Functional Anatomy of a dsRNA Trigger: Differential Requirement for the Two Trigger Strands in RNA Interference. Molecular Cell. November 2000, Vol. 6, pages 1077-1087, see entire document.	1-5 and 8-13



Further documents are listed in the continuation of Box C.



See patent family annex.

Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search

28 January 2005 (28.01.2005)

Date of mailing of the international search report

28 MAR 2006

Name and mailing address of the ISA/US

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Authorized officer

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/10343

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-111 and 114-200

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☒ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US04/10343

### BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

- I. Claims 1- 34 and 196-198, drawn to methods of performing RNAi.
- II. Claims 35-87, 89-111, 114-121, 128, 143, 147-152, 159-195, 199 and 200, drawn to siRNA molecules and modifications thereof.
- III. Claim 88, drawn to a specific composition.
- IV. Claims 112 and 113, drawn to a method of gene silencing.
- V. Claims 122-127, 129, 145, and 146, drawn to a method of reducing off target effects during RNA interference.
- VI. Claims 130-136, 144, and 153-158, drawn to a unimolecular siRNA.
- VII. Claims 137-142, drawn to a method of reducing off target effects during RNA interference comprising the utilization of a unimolecular siRNA.

Additionally, applicants must elect a conjugate or specific conjugate combination among those named in claims 17,25-27,39-41, 53-55,67-69,78-80,87, 178 and 179. Each conjugate is distinct, even though they may function similarly, since no common structural core relates one to the other.

This International Searching Authority considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The inventions do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of is drawn to a method of performing RNA interference, said method comprising exposing an siRNA to a target sequence, wherein said siRNA is comprised of a sense and an antisense strand. Lewis et al (US 2002/0132788) teach a method of performing RNAi, said method comprising exposing a siRNA (RNAi molecule) to a target nucleic acid, wherein said siRNA is comprised of a sense and an antisense strand (abstract; claims 1, 9, 10, 11). The method of RNAi taught by Lewis et al. meets the structural limitations of claim 1 and absent evidence to the contrary is therefore considered to have functionality recited therein. Therefore, there is no special technical feature

# PATENT COOPERATION TREATY

## PCT

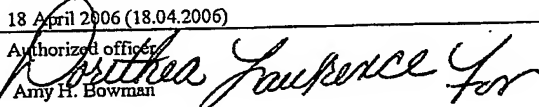
### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 08 MAY 2006

WIPO

PCT

Applicant's or agent's file reference 13510 PCT	FOR FURTHER ACTION <span style="float: right;">See Form PCT/IPEA/416</span>																									
International application No. PCT/US04/10343	International filing date (day/month/year) 01 April 2004 (01.04.2004)	Priority date (day/month/year) 02 April 2003 (02.04.2003)																								
International Patent Classification (IPC) or national classification and IPC IPC: A61K 31/70( 2006.01);C12Q 1/68( 2006.01);C07H 21/04( 2006.01) USPC: 514/44;435/6;536/24.5																										
Applicant DHARMACON, INC.																										
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of <u>6</u> sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p style="margin-left: 20px;">a. <input type="checkbox"/> (sent to the applicant and to the International Bureau) a total of ___ sheets, as follows:</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>																										
<p>4. This report contains indications relating to the following items:</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;"><input checked="" type="checkbox"/></td> <td style="width: 20%;">Box No. I</td> <td>Basis of the report</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. II</td> <td>Priority</td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td><input type="checkbox"/></td> <td>Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </table>			<input checked="" type="checkbox"/>	Box No. I	Basis of the report	<input type="checkbox"/>	Box No. II	Priority	<input checked="" type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	<input checked="" type="checkbox"/>	Box No. IV	Lack of unity of invention	<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	<input type="checkbox"/>	Box No. VI	Certain documents cited	<input type="checkbox"/>	Box No. VII	Certain defects in the international application	<input type="checkbox"/>	Box No. VIII	Certain observations on the international application
<input checked="" type="checkbox"/>	Box No. I	Basis of the report																								
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<input type="checkbox"/>	Box No. VIII	Certain observations on the international application																								
Date of submission of the demand 20 October 2004 (20.10.2004)	Date of completion of this report 18 April 2006 (18.04.2006)																									
Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer  Amy H. Bowman Telephone No. (571) 272-0755																									

Form PCT/IPEA/409 (cover sheet)(April 2005)

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/US04/10343

## Box No. I Basis of the report

1. With regard to the language, this report is based on:

- ☒ the international application in the language in which it was filed.
- ☐ a translation of the international application into English, which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
- ☐ publication of the international application (under Rule 12.4(a))
- ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

- ☒ the international application as originally filed/furnished
- ☒ the description:  
pages 1-132 as originally filed/furnished  
pages\* NONE received by this Authority on \_\_\_\_\_  
pages\* NONE received by this Authority on \_\_\_\_\_
- ☒ the claims:  
pages 133-156 as originally filed/furnished  
pages\* NONE as amended (together with any statement) under Article 19  
pages\* NONE received by this Authority on \_\_\_\_\_  
pages\* NONE received by this Authority on \_\_\_\_\_
- ☒ the drawings:  
pages 1-49 as originally filed/furnished  
pages\* NONE received by this Authority on \_\_\_\_\_  
pages\* NONE received by this Authority on \_\_\_\_\_
- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☒ The amendments have resulted in the cancellation of:

- ☒ the description, pages NONE
- ☒ the claims, Nos. NONE
- ☒ the drawings, sheets/figs NONE
- ☒ the sequence listing (*specify*): NONE
- ☒ any table(s) related to the sequence listing (*specify*): NONE

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages \_\_\_\_\_
- ☐ the claims, Nos. \_\_\_\_\_
- ☐ the drawings, sheets/figs \_\_\_\_\_
- ☐ the sequence listing (*specify*): \_\_\_\_\_
- ☐ any table(s) related to the sequence listing (*specify*): \_\_\_\_\_

\* If item 4 applies, some or all of those sheets may be marked "superseded."

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

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**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 112 and 113

because:

☐ the said international application, or the said claim Nos. \_\_\_\_\_ relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. \_\_\_\_\_ are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. \_\_\_\_\_ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 112 and 113

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/US04/10343

## Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees the applicant has, within the applicable time limit:
- ☐ restricted the claims.
  - ☐ paid additional fees.
  - ☐ paid additional fees under protest, and, where applicable, the protest fee
  - ☐ paid additional fees under protest but the applicable protest fee was not paid
  - ☐ neither restricted the claims nor paid additional fees
2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:
- ☐ complied with.
  - ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

- I. Claims 1-34, and 196-198, drawn to methods of performing RNAi.
- II. Claims 35-87, 89-111, 114-121, 128, 143, 147-152, 159-195, 199, and 200, drawn to siRNA molecules and modifications thereof.
- III. Claim 88, drawn to a specific composition.
- IV. Claims 112 and 113, drawn to a method of gene silencing.
- V. Claims 122-127, 129, 145, and 146, drawn to a method of reducing off target effects during RNA interference.
- VI. Claims 130-136, 144, and 153-158, drawn to a unimolecular siRNA.
- VII. Claims 137-142, drawn to a method of reducing off target effects during RNA interference comprising the utilization of a unimolecular siRNA.

Additionally, applicants must elect a conjugate or specific conjugate combination among those named in claims 17,25-27,39-41, 53-55,67-69,78-80,87, 178 and 179. Each conjugate is distinct, even though they may function similarly, since no common structural core relates one to the other.

The inventions do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of is drawn to a method of performing RNA interference, said method comprising exposing an siRNA to a target sequence, wherein said siRNA is comprised of a sense and an antisense strand. Lewis et al (US 2002/0132788) teach a method of performing RNAi, said method comprising exposing a siRNA (RNAi molecule) to a target nucleic acid, wherein said siRNA is comprised of a sense and an antisense strand (abstract; claims 1, 9, 10, 11). The method of RNAi taught by Lewis et al. meets the structural limitations of claim 1 and absent evidence to the contrary is therefore considered to have functionality recited therein. Therefore, there is no special technical feature.

4. Consequently, this report has been established in respect of the following parts of the international application:
- ☐ all parts
  - ☒ the parts relating to claims Nos. 1-111 and 114-200

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.  
PCT/US04/10343

## Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. Statement

Novelty (N)	Claims <u>Please See Continuation Sheet</u>	YES
	Claims <u>Please See Continuation Sheet</u>	NO
Inventive Step (IS)	Claims <u>Please See Continuation Sheet</u>	YES
	Claims <u>Please See Continuation Sheet</u>	NO
Industrial Applicability (IA)	Claims <u>Please See Continuation Sheet</u>	YES
	Claims <u>Please See Continuation Sheet</u>	NO

### 2. Citations and Explanations (Rule 70.7)

Claims 2-5 and 10-13 lack an inventive step under PCT Article 33(3) as being obvious over Fire et al. (U.S. 6,506,559). Fire et al. teach RNAi wherein the RNA may comprise one or more strands of polymerized ribonucleotides and modifications to either the phosphate-sugar backbone or the nucleoside. Fire et al. specifically teach double-stranded structures formed by a single self-complementary RNA strand or two separate complementary RNA strands. Fire et al. teach duplexes 25 nucleobases in length.

Fire et al. do not teach the specific modifications instantly claimed.

Claim 1 lacks novelty under PCT Article 33(2) as being anticipated by Fire et al. (U.S. 6,506,559). Fire et al. teach a method of RNAi involving siRNA molecules comprising a sense and antisense strand.

Claims 1-5 and 10 lack novelty under PCT Article 33(2) as being anticipated by Amarzguioui et al. Amarzguioui et al. teach 21-nt. siRNA duplexes and RNAi, wherein the siRNA strands comprise 2'-O-methylation, 2'-O-allylation or phosphorothioates. The modifications were tested at the 5' and 3' ends, as well as in the non-basepairing 3' overhangs.

Claims 1-5 and 8-13 lack novelty under PCT Article 33(2) as being anticipated by Parrish et al. Parrish et al. teach modified siRNA duplexes and the RNAi mechanism. Parrish et al. teach 2'-O-alkyl (i.e. 2'-O-methyl) modifications at various nucleotide positions of the siRNA duplex. Parrish et al. teach inverted deoxythymidine modified nucleotides and 2'-flourouracil modifications that meet the limitations of the instant claims.

Claims 1-19,34,75-86,89-111, 114-158,162,164-194,196 and 198-200 lack novelty under PCT Article 33(2) as being anticipated by Beigelman et al. (WO 02/094185). Beigelman et al. teach conjugates of biologically active compounds including antisense, dsRNA, ribozymes, and siRNA. Beigelman et al. teach conjugates that improve the bioavailability and pharmacodynamics of a molecule compared to unconjugated molecules. Beigelman et al. teach that hammerhead ribozymes have been conjugated to receptor antibodies. Beigelman et al. teach the usage of linker structures and labels. Beigelman et al. teach a wide array of conjugates including lipids, small molecules, polyethylene glycol conjugates, protector groups and PEG. Additionally, Beigelman et al. teach modification of nucleic acid molecules to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, and 2'-H groups. Beigelman et al. teach phosphorothioate, methylphosphonate inverted abasic modification. The modifications and conjugates taught by Beigelman et al. are applicable at various locations of the nucleotide. Further, siRNA molecules would inherently be involved in the RNAi mechanism.

Additionally, claims 20-33,35-74,87,88,159-161, 114-161,163,195 and 197 lack an inventive step under PCT Article 33(3) as being obvious over Beigelman et al. (WO 02/094185), in view of Scaringe et al. (U.S. 6,590,093). Although Beigelman et al. teach various modifications and conjugates of biologically active compounds including antisense, dsRNA, ribozymes, and siRNA, Beigelman et al. do not teach orthoester modifications. Beigelman et al. apply modifications and conjugates to biologically active compounds including antisense, dsRNA, ribozymes, and siRNA, as they are considered functionally equivalent.

Scaringe et al. teach orthoester protecting groups and their use with antisense oligonucleotides and ribozymes. Scaringe et al. teach that orthoester groups, as well as in combination with 2'-modifications, help minimize degradation.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.  
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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

V.1. Reasoned Statements:

The opinion as to Novelty was positive (Yes) with respect to claims 20-33, 35-74, 87, 88, 159-161, 163, 195 and 197  
The opinion as to Novelty was negative (No) with respect to claims 1-19, 34, 75-86, 89-111, 114-158, 162, 164-194, 196, and 198-200  
The opinion as to Inventive Step was positive (Yes) with respect to claims NONE  
The opinion as to Inventive Step was negative (NO) with respect to claims 1-111 and 114-200  
The opinion as to Industrial Applicability was positive (YES) with respect to claims 1-111 and 114-200  
The opinion as to Industrial Applicability was negative (NO) with respect to claims NONE